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ORIGINAL PAPER

Effects of Motilin and Mitemcinal (GM-611) on Gastrointestinal Contractile Activity in Rhesus Monkeys In Vivo and In Vitro

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Abstract Neither the presence of motilin receptors nor their action has been investigated in monkeys. The object of this study was to determine the effects of motilin and mitemcinal (GM-611), an erythromycin derivative, on the gastrointestinal tract in rhesus monkeys in vivo and in vitro. In in vivo investigations in conscious monkeys, both motilin and mitemcinal induced migrating motor complex-like contractions in the interdigestive state and also accelerated gastric emptying. In in vitro investigations, the presence of motilin receptors in the gastrointestinal tract was demonstrated by receptor binding assays. Motilin and mitemcinal contracted isolated duodenum strips in a concentration-dependent manner. In conclusion, rhesus monkeys may be useful for studying the physiological and pharmacological roles of the motilin agonistic mechanism because they show reactivity to motilin both in vivo and in vitro.

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Introduction

Motilin, a 22-amino acid residue peptide, was first characterized and isolated from porcine small intestinal mucosa [1]. Since then, motilin has been discovered in several other animals including humans. Human motilin has the same sequence as porcine motilin, and differs by three amino acid residuals from rabbit motilin and by five amino acid residuals from both canine and cat motilin [2].

It is well known that there are several species differences in the action of motilin. For example, in in vitro studies, motilin induced concentration-dependent contractions of rabbit duodenum strips and human stomach but did not induce contractions in rat or guinea pig tissues [3, 4]. Receptor binding assays of rabbit and human gastrointestinal tissue homogenates demonstrated specific binding to motilin, indicating that motilin receptors are present in the gastrointestinal tracts of these species [5, 6], but receptor binding assays of similarly prepared dog tissue homogenates did not demonstrate the presence of motilin receptors [6]. In in vivo studies, exogenous motilin induced migrating motor complex (MMC)-like contractions in dogs and humans [7, 8], but the activity of motilin in rabbits differed from that seen in dogs and humans in that although intravenous (i.v.) administration of motilin increased contractile activity in the upper gastrointestinal tract in rabbits, the contractions did not migrate distally [9].

The mode of action of motilin has not been well clarified; it is not known whether motilin acts through intrinsic neurons, extrinsic neurons, or smooth muscles; and it is likely that the

actions of motilin and its mechanisms differ under different experimental conditions (e.g., in vivo experiments, isolated strips, and isolated whole organs) even in the same animal species. For example, the spontaneous MMC and motilin-induced MMC-like contractions were completely abolished by atropine and hexamethonium in the stomachs of conscious dogs, so the final motilin mediator would seem to be acetylcholine released from the vagus nerve in the dog stomach [2]. Motilin agonist-induced MMC-like contractions in the dog stomach are also antagonized in vivo by systemic treatment with 5-HT_3 antagonists [10, 11]. It is known that the area postrema is very rich in fenestrated capillaries and has numerous neurons including 5-HT neurons in the perivascular spaces around capillaries [12], so it is generally believed that motilin agonists may stimulate motilin receptors in 5-HT neurons in the area postrema and that stimulation of 5-HT neurons activates vagal efferents through 5-HT_3 receptors [2]. So far, however, the existence of motilin receptors in the area postrema has not been demonstrated. On the other hand, isolated canine duodenum strips were not contracted by porcine motilin, and were contracted by canine motilin only when treated with nonphysiological high concentrations [13]. The contractile response to canine motilin in the canine duodenum was resistant to tetrodotoxin and atropine but was sensitive to verapamil [13]. Motilin caused phasic contractions in isolated canine stomach independently of the presence of extrinsic nerves, and these contractions were significantly inhibited by atropine and hexamethonium [14].

It has been reported that erythromycin A (EMA), a macrolide antibiotic, acts as a motilin receptor agonist [2, 15, 16]. As a result of efforts to eliminate the antimicrobial activity and enhance the motilin agonistic activity of EMA, a large number of derivatives, such as EM-523 [17], EM-574 [18], and ABT-229 [19], have been produced in the hope of finding a treatment for motility disorders such as gastroparesis [2, 16]. The preclinical studies for most of the above compounds have mainly used dogs (in vivo) and rabbits (in vitro) [16]. Because there are several differences in the actions of motilin in different species and under different experimental conditions, as described above, it may be difficult to predict the effects of EMA derivatives in humans based on preclinical results in dogs or rabbits.

The present study was conducted to test the hypothesis that motilin reactivity is similar in monkeys and humans. This hypothesis is based on the fact that the sequence of the monkey motilin has been found to be only one amino acid different from that of human motilin [20]. Since there have been no reports of motilin reactivity in monkeys, the present study was designed to investigate the actions of motilin and mitemcinal on the rhesus monkey gastrointestinal tract, both in vivo and in vitro. Mitemcinal ([2*S*,4*R*,5*R*,8*R*,9*S*,10*S*,11*R*,12*R*]-9-[(2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-ribo-hexopyranosyl)oxy]-5-ethyl-4-methoxy-2,4,8,10,12,14-hexamethyl-11-[[3,4,6-trideoxy-3-(isopropylmethylamino)-β-D-xylo-hexopyranosyl]oxy]-6,15-dioxabicyclo[10.2.1]pentadec-14(1)-ene-3,7-dione(*E*)-2-butenedioic acid salt [2:1]; code name, GM-611) is an EMA derivative that was synthesized in our laboratory [21]. It was reported that mitemcinal acted as a selective and full motilin receptor agonist in the smooth muscle of the rabbit small intestine [22]. Clinical trials of mitemcinal in patients with diabetic gastroparesis are currently under way [23, 24].

Materials and methods

Animals

Male rhesus monkeys (approximately 5 kg; Chugai Research Institute for Medical Science, Inc., Shizuoka, Japan) and male Japanese white rabbits/CSK (approximately 2–3 kg; Chugai Research Institute for Medical Science) were used in this study. The monkeys were individually housed in experimental cages and given monkey diet (PS, 120 g/body; Oriental Yeast Co., Tokyo) and one piece of banana once a day at 4:00 PM. All animal procedures were conducted in accordance with Chugai Pharmaceutical's ethical guidelines for animal care, and all experimental protocols were approved by the Animal Care Committee of the institution.

In vivo investigations

Measurement of gastrointestinal contractile activity in conscious Monkeys

Animals were anesthetized with an intramuscular injection of ketamine (15 mg/kg; Sankyo-Yell Yakuhin Co., Tokyo) and an i.v. injection of pentobarbital (15 mg/kg; Abbott Labs, Chicago, IL). Prior to the start of surgery, atropine (50 μg/kg; Tanabe Seiyaku, Osaka, Japan) was injected i.v. to prevent salivation and gastrointestinal motility. The abdomen was opened and force transducers (F-12IS [8 × 13 mm] and F-8IS [5 × 8 mm]; Star Medical Inc., Tokyo) were sutured at the following locations in orientations that enabled measurement of circular muscle contractions: the gastric antrum (F-12IS; 3 cm proximal to the pyloric ring), the duodenum (F-8IS; 3 cm distal from the pyloric ring), and the jejunum (F-8IS; 30 cm distal from the pyloric ring). After the above abdominal surgery, a silicon tube (602-205; Dow Corning, Midland, MI) for i.v. administration of drugs was implanted into the vena cava via the right external jugular vein, and the lumen of the tube was filled with heparinized 0.9% saline solution.

After a 2- to 3-week recovery period, the gastrointestinal contractile activity in the conscious monkeys was recorded using a thermal pen recorder (WR3701; Graphtec Corp.,

Tokyo) through an Itoh-type amplifier (SS-1786; Nihon Kohden, Tokyo) by connecting cable lead wires from the amplifier to the lead wires of the force transducers. The contractile activity signal from the gastric antrum was also input to a signal processor (7T; Nihon Kohden/NEC San-Ei Instruments, Tokyo), and quantitative analysis was performed by calculating the motor index (MI) as the area between the contractile wave and the baseline. The MI of the gastric antrum was evaluated based on the assumption that the contraction area is 100 when maximal phase III contractions of the MMC continue for 1 min [25].

Porcine motilin (Sigma Chemical Co., St. Louis, MO) and mitemcinal (synthesized in our organic laboratory) were dissolved in 0.9% saline and administered i.v. about 15 min after the end of the MMC cycle in the gastric antrum via the silicon tube implanted into the vena cava. To examine the effects of several blockers on the contractile activity induced by porcine motilin, GM-109, a selective motilin receptor antagonist synthesized in our laboratory [26], was administered by continuous i.v. infusion from 5 min prior to 10 min after porcine motilin administration at a rate of 0.25 μmol/kg/min, and atropine (0.3 μmol/kg, a muscarinic antagonist) and hexamethonium (10 μmol/kg, a nicotinic antagonist; Sigma Chemical) were injected i.v. 5 min prior to the administration of porcine motilin. The effective dose of atropine used in this study has been confirmed to be appropriate for examining the effect of atropine on contractile activities induced by i.v. administration of bethanechol (dissolved in 0.9% saline; 3 μmol/kg; Sigma Chemical).

Measurement of the gastric emptying rate in conscious monkeys

Before the start of each experiment, the animals were acclimated to the oral administration procedure to avoid stress caused by the administration method. That is, each animal was restrained on a monkey chair and a feeding tube (6 Fr Atom Medical Co., Tokyo) was inserted into its stomach from the nose every day for 7 days. The gastric emptying rate of monkeys was measured by the acetaminophen (AAP) method [27] after overnight fasting. The monkey was restrained in a monkey chair and 10 ml/kg liquid meal (Okunos Liquid Food A; 14.5% carbohydrate, 5.1% protein, 2.8% lipid; 240 J/ml; Forica Foods Co., Niigata, Japan) thoroughly mixed with 40 mg/ml AAP (Wako Pure Chemical Industries, Osaka, Japan) was administered orally via the transnasal feeding tube. Blood samples (0.5 ml) were collected with a heparinized syringe from the cubital vein every 15 min from 0 to 90 min, and every 30 min from 90 to 180 min, after administration of the liquid meal. The concentration of AAP in the plasma of the blood samples was measured by HPLC. The peak plasma concentration, time to peak concentration, and AUC_{0-60} of AAP were calculated. Porcine motilin, mitemcinal, or 0.9% saline as the corresponding vehicle was administered i.v. 5 min after administration of the liquid meal. In each animal each experiment was conducted at intervals of at least 1 week.

In vitro investigations

Motilin receptor binding assay using gastrointestinal tissue homogenates

The binding investigation was performed using the method described in a previously reported study in rabbits [5], with minor modifications. Each monkey was anesthetized with ketamine and pentobarbital, as described above, and euthanized by exsanguination via the carotid artery. After exsanguination, the gastrointestinal tract of the monkey was rapidly removed, rinsed with ice-cold 0.9% saline, and separated into various gastrointestinal tract regions, as follows. The stomach was divided into three regions from the proximal to the distal end: the fundus, the corpus, and the antrum. The small intestine (total length, about 1.5 m) was divided into three regions from the proximal to the distal end: the duodenum, the jejunum, and the ileum. For the colon (total length, about 70 cm), 50 cm of the proximal side was used in the study. Connective tissue and the mucosal layer were removed from each segment, and the remaining tissue was homogenized in 50 mM Tris-HCl buffer (pH 7.4) at 0°C using a tapered homogenizer (Model 358115; Wheaton Science, Millville, NJ). The homogenate was centrifuged (TMP-22; Hitachi Koki Co., Ltd., Tokyo) at 1500*g* for 5 min and then washed twice with fresh Tris-HCl buffer (50 mM, pH 7.4). The final pellet was resuspended in 50 mM Tris-HCl buffer (pH 8.0; containing 10 mM $MgCl_2$ and 1.5% bovine serum albumin) for the binding investigation. The homogenate was incubated at 25°C for 120 min with 25 pM ^{125}I-porcine motilin (Peninsula Laboratories Inc., Belmont, CA) made up to 1 ml. After incubation, the reaction was stopped by adding 2 ml of ice-cold buffer. Bound and free reagents were separated by centrifugation at 1500*g* for 5 min. The pellet was washed with ice-cold buffer, and its radioactivity was determined using a gamma counter (ARC-300; Aloka, Tokyo).

Specific binding was defined as the difference between total and nonspecific binding after the addition of 1 μM porcine motilin. First, the ratio of specific to total binding of ^{125}I-porcine motilin in each region of the gastrointestinal tract was examined. Second, displacement curves were obtained by adding increasing amounts of porcine motilin, canine motilin (Sigma Chemical Co.), mitemcinal, or GM-109 to the duodenum tissue incubation sample, and the concentrations that reduced specific binding by 50% (IC_{50}) were found. All of the procedures, from isolation of the tissue to incubation, were performed under ice-cold conditions.

To compare the affinities of motilin ligands for motilin receptors in monkey and rabbit tissue, displacement curves for porcine motilin, mitemcinal, and GM-109 were also obtained using rabbit duodenal tissue homogenates prepared by the above method.

Contractile activity in isolated monkey duodenal muscle strips

Monkeys were anesthetized and euthanized by the above method. The upper part of the small intestine was rapidly removed after laparotomy and placed in ice-cold modified Krebs' solution composed of (mM) 120.0 NaCl, 4.7 KCl, 2.4 $CaCl_2$, 1.0 KH_2PO_4, 1.2 $MgSO_4$, 24.5 $NaHCO_3$, and 5.6 glucose (pH 7.4). The duodenum was washed, freed from mesenteric attachment, and cut along the longitudinal axis to obtain muscle strips about 10 mm long and 3 mm wide. The strips were then mounted in an organ bath containing 10 ml of modified Krebs' solution kept at 28°C. The solution was gassed with a mixture of 95% O_2 and 5% CO_2. The longitudinal strips were initially loaded with a 1.0-g weight, and contractile activity was measured by isotonic transducers (ME-4012; Medical Electronics Co., Tokyo) and recorded on an ink-writing recorder (Type 3066; Yokogawa-Denki, Tokyo). Before the experiments, each strip was subjected to repeated stimulation with 100 μM acetylcholine (Dai-ichi Pharmaceutical Co., Tokyo) until a reproducible response was obtained, and then porcine motilin or mitemcinal was added cumulatively to the organ bath. To normalize the data, the contractile potency of each agonist was expressed as a percentage of that induced by 100 μM acetylcholine. To examine the influences of atropine and verapamil (Ca channel blocker; Sigma Chemical Co.) on the contraction induced by porcine motilin, isolated duodenum strips were treated for 15 min with either atropine (1 μM) or verapamil (1 μM). The contractile response induced by porcine motilin (30 nM) in the absence or presence of each blocker was compared. All drug concentrations are expressed as final molar concentrations in the bath solution.

Statistical analysis

All data are expressed as the mean ± SE. Statistical analysis was performed using Dunnett's multiple-comparison test. P values <0.05 were considered statistically significant.

Results

Spontaneous gastrointestinal contractile activity in conscious monkeys

Typical tracings of spontaneous gastrointestinal contractile activity patterns are shown in Fig. 1. In the interdigestive state, MMC occurred regularly in the gastric antrum and duodenum and then migrated to the jejunum. After feeding, the contractile activity pattern changed to the digestive state, that is, MMC disappeared and small irregular contractions occurred in the gastric antrum, duodenum, and jejunum. The spontaneous MMC pattern in the interdigestive state was measured for 10 days in four animals. MMC lasting 25 ± 1 min started at 928 ± 25 min after feeding and recurred every 120 ± 5 min. The mean MI of the gastric antrum was 158.3 ± 13.5. The observational results are summarized in Table 1.

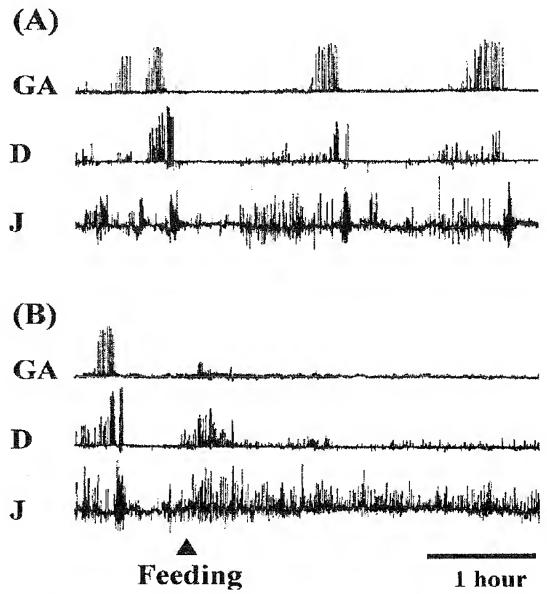

Fig. 1 Typical tracings of gastrointestinal contractile activity in the interdigestive state (A) and the digestive state (B) in a conscious monkey. GA, gastric antrum; D, duodenum; J, jejunum. Horizontal bar shows 1 hr

Effects of drugs on gastrointestinal contractile activity in conscious monkeys in the interdigestive state

Intravenous injection of porcine motilin (≥0.3 nmol/kg) or mitemcinal (≥30 nmol/kg) induced MMC-like contractions similar to the spontaneous MMC in the upper gastrointestinal tract (Figs. 2A and B). The MI of the gastric antrum was increased by porcine motilin and mitemcinal in a dose-dependent manner (Fig. 2C). The gastric contractile activity induced by porcine motilin was completely inhibited by continuous i.v. infusion of GM-109 (Fig. 3A). The contractile activity induced by porcine motilin was significantly inhibited by pretreatment with hexamethonium but not by pretreatment with atropine (Fig. 3A). The contractile activity induced by bethanechol was not inhibited by hexamethonium but was completely abolished by atropine (Fig. 3B).

Table 1 Spontaneous contractions in conscious monkeys: mean ± SE (minimum–maximum)

Monkey No.	Duration of feeding stage (min)	MMC interval (min)	MMC duration (min)	MMC MI in the GA
1	938 ± 29 (784–1086)	152 ± 14 (39–268)	25 ± 2 (12–51)	176.7 ± 33.6 (73.4–381.5)
2	922 ± 63 (480–1140)	112 ± 11 (41–328)	19 ± 1 (8–33)	192.3 ± 33.7 (40.0–381.6)
3	1008 ± 27 (841–1158)	110 ± 11 (54–251)	28 ± 2 (11–69)	103.8 ± 9.9 (44.5–143.0)
4	844 ± 58 (436–1129)	116 ± 6 (42–205)	27 ± 1 (11–62)	160.1 ± 11.2 (113.2–231.0)
Average	928 ± 25 (436–1158)	120 ± 5 (39–328)	25 ± 1 (8–69)	158.3 ± 13.5 (40.0–381.6)

Note. Duration of feeding stage (min): time from feeding to the start of the first MMC cycle in the gastric antrum. MMC interval (min): time between two MMC cycles in the gastric antrum, measured from the start of one cycle to the start of the next cycle. MMC duration (min): duration of each MMC cycle in the gastric antrum. MMC MI in the GA: the motor index of the MMC in the gastric antrum.

Effects of porcine MTL and mitemcinal on the gastric emptying rate in conscious monkeys

Porcine motilin (0.1–1 nmol/kg) and mitemcinal (30–300 nmol/kg) given i.v. 5 min after ingestion of a liquid meal thoroughly mixed with AAP dose dependently decreased the time to peak AAP concentration, increased the peak AAP concentration, and increased the AUC_{0-60} of AAP (Fig. 4 and Table 2).

Motilin receptor binding assay in monkeys

Preliminary to the study, the distribution of motilin receptors in the gastrointestinal tract was examined by assay of each region. The ratio of specific to total binding of ^{125}I-porcine motilin in each region is shown in Fig. 5A. Specific binding of motilin was observed in the gastric antrum, duodenum, jejunum, and ileum but was not confirmed in the gastric fundus, corpus, or colon. The highest ratio of specific to total binding of motilin was observed in the duodenum, so the duodenal muscle tissue was used in the following investigation.

The effects of several motilin ligands on the binding of ^{125}I-porcine motilin to monkey duodenal homogenate were investigated. The displacement curves obtained by adding ^{125}I-porcine motilin and increasing concentrations of porcine motilin, canine motilin, mitemcinal, and GM-109 to monkey duodenal tissue are shown in Fig. 5B; all of these

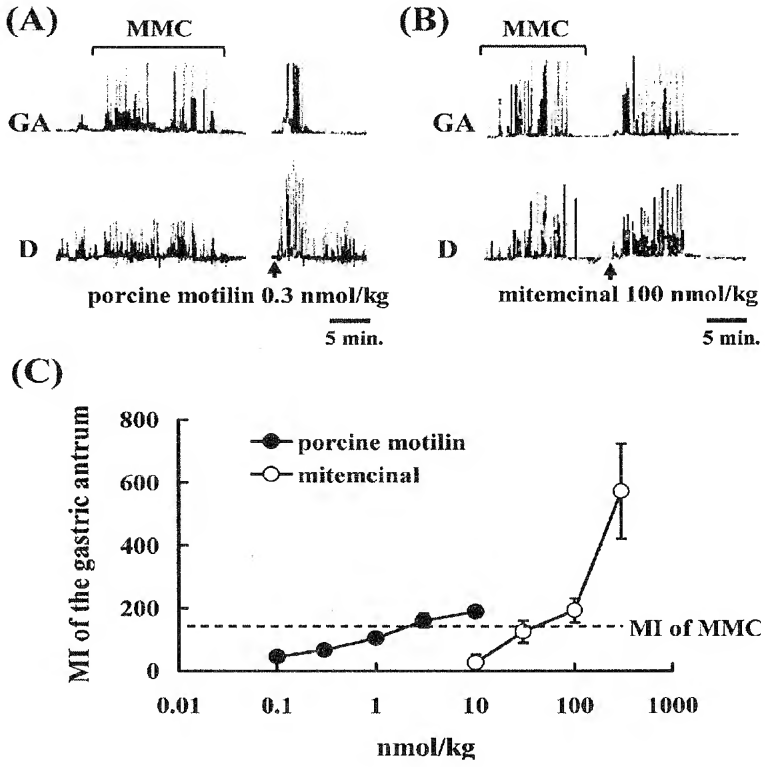

Fig. 2 Effects of i.v. administration of (A) porcine motilin (0.3 nmol/kg) or (B) mitemcinal (100 nmol/kg) on gastrointestinal contractile activity in conscious monkeys. GA, gastric antrum; D, duodenum. Horizontal bar shows 5 min. (C) Dose-response curves for i.v. injection of porcine motilin (●; 0.1–10 nmol/kg) or mitemcinal (○; 10–300 nmol/kg) on the motor index (MI) of the gastric antrum in conscious monkeys in the interdigestive state. Each point represents the mean ±SE of four animals. The dotted line shows the mean MI of the gastric antrum from the spontaneous MMC of four animals

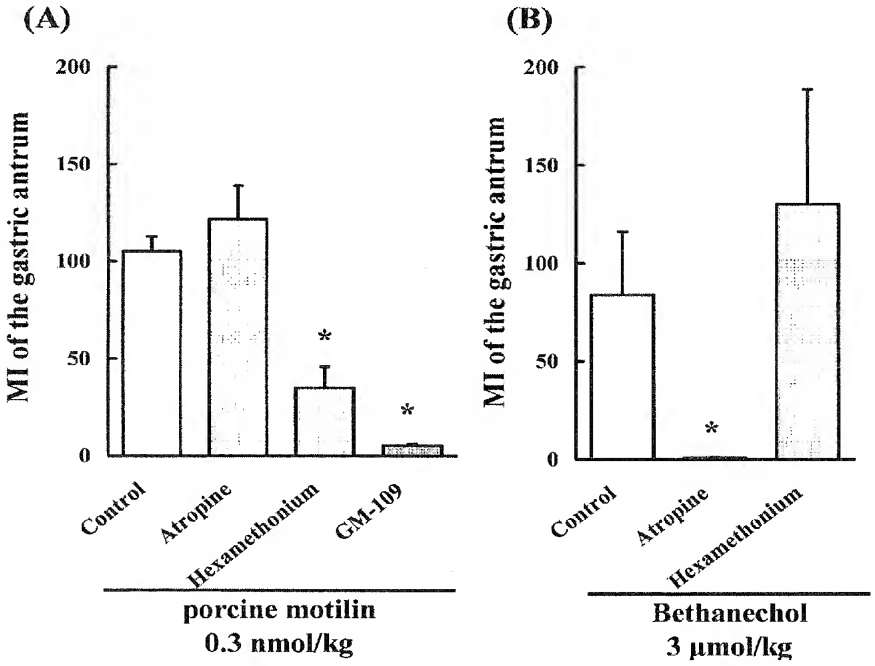

Fig. 3 Effects of atropine, hexamethonium, and GM-109 on the motor index (MI) of the gastric antrum induced by porcine motilin (A; 0.3 nmol/kg) or bethanechol (B; 3 μmol/kg) administered i.v. to conscious monkeys. Each column represents the mean ±SE of three or four animals. Atropine (0.3 μmol/kg) or hexamethonium (10 μmol/kg) was injected i.v. 5 min before the administration of porcine motilin or bethanechol. GM-109 (0.25 μmol/kg/hr) was given by continuous i.v. infusion from 5 min before to 10 min after the administration of porcine motilin. $^*P < 0.05$ compared with each agonist alone (control) group by Dunnett's test

curves are parallel. The IC_{50} values for porcine motilin, canine motilin, mitemcinal, and GM-109 are shown in Table 3.

Motilin receptor binding assay in rabbits

The displacement curves for porcine motilin, mitemcinal, and GM-109 in the motilin receptor binding investigation using rabbit duodenal tissue were also parallel (data not shown). The IC_{50} values are summarized in Table 3.

Effects of drugs on the contractile activity in isolated monkey duodenal strips

Porcine motilin (1–1000 nM) and mitemcinal (30–10,000 nM) induced contractions in isolated duodenal longitudinal muscle strips of monkeys in a concentration-dependent manner (Figs. 6A–C). The maximum contractions of duodenal strips caused by porcine motilin and mitemcinal were 72.3% ± 4.1% and 53.8% ± 6.5% (percentages of the contraction caused by 100 μM acetylcholine; both $Ns = 8$) (Fig. 6C). The contractions induced by both agonists were

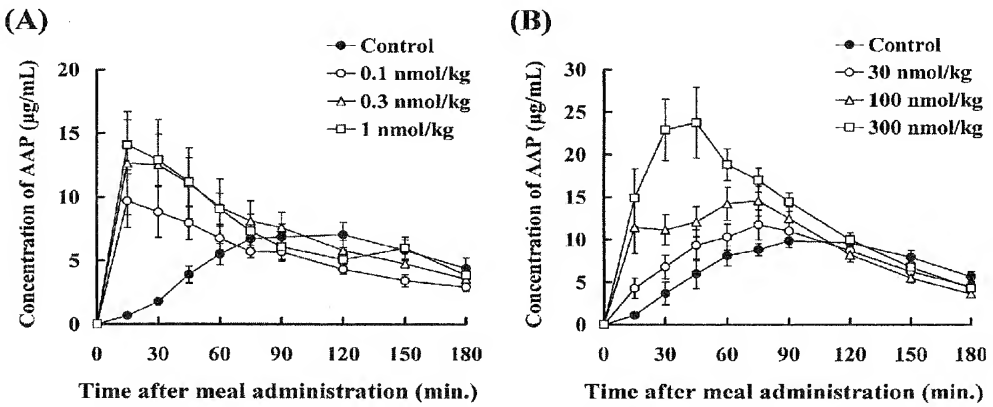

Fig. 4 Changes in plasma concentration of AAP (μg/ml) after oral administration of a liquid meal (10 ml/kg) containing 40 mg/ml AAP. (A) Porcine motilin (○, 0.1 nmol/kg; △, 0.3 nmol/kg; □, 1 nmol/kg) or (B) mitemcinal (○, 30 nmol/kg; △, 100 nmol/kg; □, 300 nmol/kg) was administered i.v. 5 min after the liquid meal administration. As the corresponding vehicle, 0.9% physiological saline (●) was administered i.v. Each point represents the mean ± SE of six animals

Table 2 Peak concentration, time to peak concentration, and AUC_{0-60} of acetaminophen (AAP) in conscious monkeys after administration of a liquid meal containing 40 mg/ml AAP

Drug	Dose (nmol/kg)	Peak concentration of AAP (μg/ml)	Time to peak concentration (min)	AUC_{0-60} (μg · min/ml)
Porcine motilin	Vehicle	7.5 ± 1.2	105.0 ± 9.5	136.2 ± 19.7
	0.1	10.4 ± 2.0	30.0 ± 10.2*	446.7 ± 93.8
	3	14.7 ± 4.2	48.5 ± 18.7*	612.1 ± 184.9*
	1	13.8 ± 2.0	25.0 ± 7.4*	586.5 ± 72.9*
Mitemcinal	Vehicle	10.3 ± 1.0	92.5 ± 11.2	222.1 ± 60.5
	30	12.0 ± 1.7	85.0 ± 7.4	379.6 ± 75.7
	100	15.4 ± 1.4	52.5 ± 12.1	556.9 ± 75.0
	300	27.2 ± 4.3*	37.5 ± 8.4*	1064.7 ± 137.3*

Note. Each value represents the mean ± SE of six observations in six animals. Porcine motilin, mitemcinal, or 0.9% saline as the corresponding vehicle was administered i.v. 5 min after ingestion of the liquid meal. *$P < 0.05$ compared with the corresponding vehicle group by Dunnett's test.

diminished by the addition of 10 μM GM-109 (Figs. 6A and B). The contractions induced by porcine motilin were not affected by pretreatment with 1 μM atropine but were completely inhibited by pretreatment with 1 μM verapamil (Fig. 6D).

Discussion

The findings of the present study were that (i) spontaneous MMC similar to those in dogs and humans occurred in conscious monkeys in the interdigestive state, and (ii) motilin and mitemcinal (an EMA derivative) induced contractions in the gastrointestinal tract of monkeys, both in vivo and in vitro, as in humans. To the best of our knowledge, this is the first report of the motilin-induced gastrointestinal contractile activity in monkeys.

In the present study, two gastrointestinal contractile activity patterns were observed in conscious monkeys: an interdigestive pattern and a digestive pattern. These patterns were very similar to those seen in dogs [7] and humans [8]. Porcine motilin and mitemcinal administered i.v. to conscious monkeys in the interdigestive state dose dependently induced strong contractions similar to the spontaneous MMC in the gastric antrum and duodenum. The contractions induced by porcine motilin were completely abolished by continuous i.v. infusion of GM-109, indicating that porcine motilin induces gastrointestinal contractions via motilin receptors in conscious monkeys. It is clear that motilin regulates spontaneous MMC in dogs and humans because the peak plasma endogenous motilin concentration coincides with the end of the MMC cycle in the stomach and duodenum in both species [2]. Many investigators have also reported that exogenous administrations of motilin and EMA induced MMC-like contractions in the stomach and duodenum of dogs and humans [15, 16, 28]. Taken together, the results of the present study may suggest that motilin regulates the MMC cycle in the interdigestive state in conscious monkeys, as in dogs and humans.

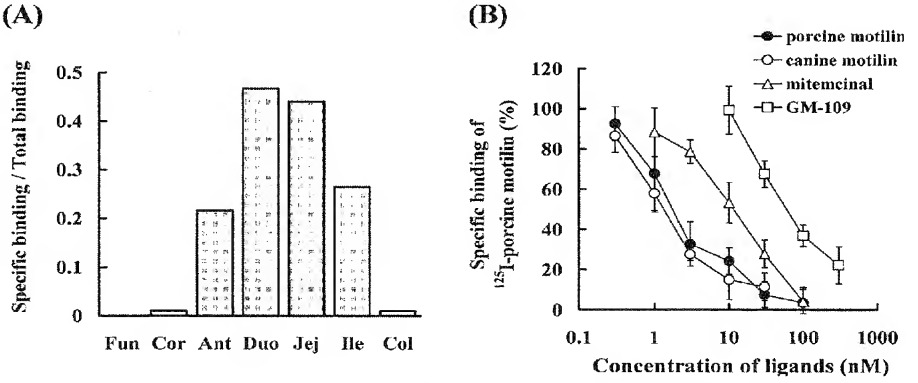

Fig. 5 (A) Distribution of motilin receptors along the gastrointestinal tract in rhesus monkeys. Binding experiments were performed with homogenates of the gastric fundus (Fun), corpus (Cor), antrum (Ant), duodenum (Duo), jejunum (Jej), ileum (Ile), and colon (Col). Each column represents the mean of two experiments. (B) Displacement of ^{125}I-porcine motilin binding in monkey duodenal tissue by porcine motilin (●), canine motilin (○), mitemcinal (△), and GM-109 (□). Each point represents the mean ± SE of three to five experiments

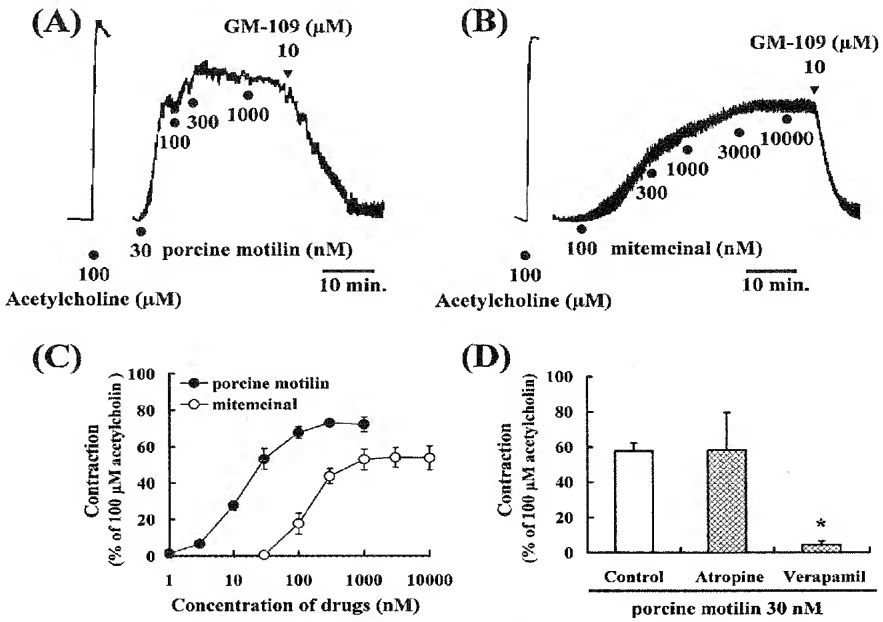

Fig. 6 Typical tracings of contractions induced by (A) porcine motilin and (B) mitemcinal in isolated monkey duodenal longitudinal muscle strips. Numbers show the molar concentration of each drug in the organ bath. Horizontal bar shows 10 min. (C) Concentration-response curves for porcine motilin (•; 1–1000 nM) and mitemcinal (○; 30–10,000 nM) in isolated monkey duodenal longitudinal muscle strips. The ordinate scale represents the percentage of the contraction induced by 100 μM acetylcholine. Each point represents the mean ± SE of eight strips. (D) The effects of atropine (1 μM) and verapamil (1 μM) on the contraction induced by porcine motilin (30 nM) in isolated monkey duodenal longitudinal muscle strips. Each column represents the mean ± SE of three to five strips. $^*P < 0.05$ compared with porcine motilin alone (control) group by Dunnett's test

It has been reported that motilin agonist-induced MMC-like contractions in the stomach are atropine-sensitive in conscious dogs. It is therefore hypothesized that the final mediator of motilin in the dog stomach is acetylcholine released from the vagus nerve [2]. In humans, motilin-induced gastric contraction was atropine-sensitive but motilin-induced duodenal contraction was atropine-resistant [29]. In the present study, surprisingly, gastric contractions induced by porcine motilin in conscious monkeys were not inhibited by atropine. The dose of atropine used in the present study was considered sufficient to inhibit the muscarinic mechanism because contractions induced by bethanechol (a cholinomimetic) were completely inhibited by the same dose of atropine. Motilin may induce gastric contractions in conscious monkeys directly via motilin receptors in the smooth muscle of the stomach. The existence of motilin receptors in the gastric antrum of monkeys was confirmed in the present binding investigation. However, the contraction induced by porcine motilin was significantly inhibited by hexamethonium, indicating that the contractile mechanisms of porcine motilin in the stomach of monkeys also involve a neuronal pathway. The results therefore suggest that the mechanisms of motilin activity in conscious monkeys act via two different pathways.

Table 3 IC_{50} values for motilin receptors in monkey and rabbit duodenal tissue homogenates

Ligand	IC_{50} value (nM) Monkey	(*N*)	Rabbit	(*N*)
Porcine motilin	2.8 ± 1.4	(5)	0.9 ± 0.1	(5)
Canine motilin	1.6 ± 0.5	(3)	Not tested	
Mitemcinal	14.8 ± 10.1	(5)	4.9 ± 0.9	(5)
GM-109	65.5 ± 7.7	(4)	10.8 ± 0.1	(5)

Note. Each value represents the mean ± SE.

It has been demonstrated that motilin receptors exist in the gastrointestinal neural and muscle layers in humans and rabbits. Motilin receptors in nerves and smooth muscle have different affinity to some motilin ligands, suggesting that motilin receptors may belong to different receptor subtypes [30, 31]. In isolated rabbit stomach, low doses of motilin (0.1–10 nM) enhanced contractions induced by electric stimulation via motilin receptors in the nerves in an atropine-sensitive manner, whereas high doses of motilin (>10 nM) induced contractions directly by activation of motilin receptors in smooth muscle in an atropine-resistant manner [32]. To identify the mechanism by which motilin induces gastrointestinal contraction in monkeys, it would be desirable to further examine the influence of several pharmacological antagonists on the responses induced by different doses of motilin.

Because motilin and mitemcinal induced gastrointestinal contractions in conscious monkeys, the effects of motilin and mitemcinal on the gastric emptying rate were investigated in the present study using an AAP method. It is known that AAP is not absorbed from the stomach but is absorbed rapidly from the small intestine, so its blood concentration is well correlated with the gastric emptying rate [27]. The present study demonstrated that porcine motilin and mitemcinal administered i.v. in a similar dose range to induce the gastrointestinal contractile activity dose dependently decreased the time to peak AAP concentration, increased the peak AAP concentration, and increased the AAP AUC_{0-60}. These results suggest that porcine motilin and mitemcinal accelerated the gastric emptying rate by increasing gastrointestinal contractile activity via a motilin agonistic mechanism. On the other hand, it is known that increased gastric motility does not necessarily lead to an accelerated gastric emptying rate because it has been reported that intraduodenal administration of a high dose of cisapride (3 mg/kg) to dogs increased gastric antral motor activity but delayed gastric emptying [33]. It is considered that well-coordinated gastrointestinal contractions within the gastric antrum and the duodenum may be important for the acceleration of gastric emptying [33]. The results of this study suggest that a motilin agonistic mechanism may induce physiological gastrointestinal contractile activity that accelerates gastric emptying in monkeys.

In the present study, specific binding of motilin was demonstrated in the gastric antrum, duodenum, jejunum, and ileum but not in the gastric fundus, corpus, or colon. Although the quantitative analysis was not sufficient, the motilin receptor density seems to be highest in the duodenum. The presence of motilin receptors has been confirmed in several animals, including rabbits [5], cats [34], and humans [6], but a motilin receptor binding study did not demonstrate the presence of motilin receptors in dogs [6]. The distribution of motilin receptors in the upper gastrointestinal tract in monkeys was similar to that previously reported in rabbits, cats, and humans [5, 6, 34]. Those reports demonstrated that motilin receptor densities in the stomach and the small intestine, respectively, are highest in the gastric antrum and the duodenum and that the motilin receptor density in the small intestine decreases aborally. There are, however, species differences in relation to the colon: in cats, no motilin receptor was detected in the colon, whereas in rabbits, motilin receptors were about four times denser in the colon than in the duodenum [35]. The results of the present study indicate that the distribution of motilin receptor in monkeys is closer to that seen in cats than to that seen in rabbits.

The present study also demonstrates that several different types of motilin ligands such as porcine and canine motilins (poly-amino acid peptides), GM-109 (a cyclic tetrapeptide), and mitemcinal (an EMA derivative) bind to motilin receptors in the duodenal muscle tissue of monkeys. The order of affinities of these ligands was consistent with those found in the rabbit motilin receptor binding investigation in this study. It seems that the binding of motilin receptors to several types of motilin ligands in monkeys is similar to that in rabbits. However, the present study also revealed several differences between monkeys and rabbits with respect to the IC_{50} values of motilin ligands. The IC_{50} value for porcine motilin in monkey tissue was 2.8 $\pm$ 1.4 nM, which is about three times higher than in rabbit tissue (0.9 $\pm$ 0.1 nM) at the same ^{125}I-porcine motilin concentration. Higher IC_{50} values were seen in monkey motilin receptors, not only for porcine motilin but also for mitemcinal and GM-109, indicating that the affinity of motilin ligands to motilin receptors is lower in monkeys than in rabbits. Differences between rabbits and humans with respect to the affinity of motilin ligands to motilin receptors have also been reported previously. The K_d value of motilin receptors in human gastric antrum smooth muscle (1.8 nM) was about three times higher than in rabbits (0.53 nM) [36]. Taken together, it seems that monkey and human motilin receptors have weaker affinities to motilin ligands than rabbit motilin receptors, indicating that the characteristics of motilin receptors may be similar in monkeys and in humans.

In the present study, porcine motilin and mitemcinal also induced contractions in isolated monkey duodenum strips in a concentration-dependent manner. These contractions were inhibited by treatment with GM-109, indicating that porcine motilin and mitemcinal induced contractions of the duodenum strips via motilin receptors. It has been reported that motilin-induced contractions in human or rabbit in vitro specimens were not affected by atropine, tetrodotoxin, or hexamethonium but were inhibited by verapamil or by removing Ca^{2+} from the medium [3, 4]. The effect of tetrodotoxin on contractions induced by porcine motilin was not tested, but the results of the present study confirm that, as in humans and rabbits, motilin-induced contractions in the monkey duodenum were atropine-resistant and verapamil-sensitive.

The maximum contractions induced by porcine motilin and mitemcinal, respectively, in isolated monkey duodenal muscle strips were 72.3% $\pm$ 4.1% and 53.8% $\pm$ 6.5% of the contraction induced by 100 μM acetylcholine (both Ns = 8). In rabbit duodenal muscle strips, however, both porcine motilin and mitemcinal induced maximal contractions 100% of that induced by 100 μM acetylcholine [22, 37]. Furthermore, the concentrations of porcine motilin and mitemcinal which induced maximal contractions in the monkey duodenal muscle strips were about 10 times higher than those that induced maximal contractions in the rabbit duodenal strips [22, 37]. Depoortere et al. compared the effect of porcine motilin and EM-523 in human and rabbit duodenal strips [36]. According to them, both porcine motilin and EM-523 induced contractions in human and rabbit duodenal muscle strips in a concentration-dependent manner, but

the concentrations of porcine motilin and EM523 required to induce maximal contraction were about 10–100 times higher for human tissue than for rabbit tissue. They also reported that porcine motilin and EM-523 induced smaller maximal contractions than acetylcholine in human strips. It is therefore considered that the contractions caused by the motilin agonistic mechanism in monkeys in vitro may resemble those seen in humans more closely than those seen in rabbits.

The present study was conducted to test the hypothesis that motilin reactivity is similar in monkeys and humans. In conclusion, the study results demonstrated that monkeys react to motilin both in vivo and in vitro, and that motilin induces contractions in the monkey gastrointestinal tract that are the same as in humans, both in vivo and in vitro. The only difference between monkeys and humans observed in the present study was that the contraction induced by porcine motilin in conscious monkeys was not suppressed by atropine. Although it would be desirable to identify the detailed mechanisms of the action of motilin in monkeys, the present study has demonstrated that the monkey is useful for the study of the pharmacological and physiological roles of motilin and motilin-related compounds because it shows reactivity to motilin both in vivo and in vitro.

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